A COMPARISON OF METHODS FOR THE EXAMINATION OF WATER AT FILTRATION PLANTS

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It is customary to compare water purification plants on the basis of bacteriologic examinations of raw and treated waters, although the raw water supplies are varied in character and filters of many kinds operated under diverse conditions. Two sorts of results are used: (a) the percentage removal of bacteria during the purifying process as estimated by the ordinary plating method or by determinations of some specific organism as B. coli; and (b) the bacterial character of the treated water regardless of its original micro-organic content. Any comparison by percentage removal is unsatisfactory because the results so largely depend on the number of bacteria in the raw water. It is relatively easy to obtain a 99% "purification" on a water containing 20,000 organisms per c c, leaving 200 still in the filtered supply, and correspondingly difficult to obtain a count of 2 from a raw water containing 200 bacteria per c c — a condition necessary to attain in order to have the same percentage reduction.

But whatever method is chosen for reporting filtration results either for comparing different system of purification or for controlling the process in a single plant, this method is dependent on the procedures in use for obtaining the bacteriologic data. There is great diversity of opinion among bacteriologists as to the most reliable procedures. Although there exists a "Standard Methods of Water Analysis," those in charge of water-works laboratories believe that it is often wise to deviate from these suggested standards.

In order to obtain some definite information concerning the bacteriologic technic in vogue in collecting data on filter plant operation, a series of questions was sent to a selected list of cities where purification works had been in operation for some years. Twenty-four satisfactory replies were received. I am indebted for the material here discussed to those so kindly responding to my request for information. The list contains plants of varying size and type, and includes 6 slow sand filters, 17 rapid filters and one in which coagulation and sedimentation are used but no filtration process. In 6 hypochlorite of lime

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is applied to the filtered water, 13 utilize liquid chlorin and one uses hypochlorite and ammonia. In 4 no disinfectant is used. The plants are widely distributed over the country and although fewer in number than desired, represent current American practice as nearly as possible.

An examination of the data received has revealed some interesting conditions which are here reported. Perhaps the most striking is that no two of the 24 laboratories use procedures for their bacterial water examinations which are identical in every respect. Certainly comparisons of analytical data under such a state of affairs is, to say the least, unfortunate.

For a detailed study the data may be grouped under 3 headings: (1) total counts on gelatin; (2) total counts on agar, and (3) tests for the presence of organisms of the colon bacillus type. To this has been added another section (4) discussing the relation of the gelatin and agar counts. In only one of the laboratories reporting is the direct microscopic method used, and here only on the raw water as supplementary to mediums.

1. GELATIN COUNTS

Of the 24 laboratories, 15 (62.5% *) employ gelatin for a total count at 20°. In one case agar medium is also used at this temperature and the results reported in the annual statements in preference to the gelatin count. In all but one instance the count is made after 48 hours at 20 C., and in the one exception the time is reported as "48 to 72 hours." The gelatin medium, as far as could be ascertained, is made to conform closely to that suggested in "Standard Methods."

In 12 of the above mentioned 15 laboratories counts are also made on agar at some higher temperature, but in two of these cases no results are reported, so that for practical statistical purposes only 10 laboratories (41.5% of the total) use both gelatin and agar at different temperatures to obtain reportable results of the number of bacteria in the raw and treated waters.

In general, the methods involving the use of a gelatin medium are fairly consistent and the results correspondingly comparable.

2. AGAR COUNTS

Twenty-one laboratories use agar at some temperature for a bacterial count, and with one exception both raw and treated water are examined. The following table shows the different periods of time allowed to elapse and the corresponding temperatures employed.

* The total number of cases is too small to allow arguments on a percentage basis. This is calculated in only a few instances and the figures used with reserve.

TABLE 1

AGAR COUNTS

Time in Hours	Temperature	Number
24	37C*	11
24 and 48	37	1
48	37	1
48 and 24	20 and 37	2
48 and 24	22 and 37	1
48	22	1
72	20	1
72	30	1
48	40	1
24 to 48	38	1
Total		21

Percentage using agar, 87.5%; percentage using agar, 24 hours at 37 C., 58.5%.
* Temperatures reported as 37 or 37.5 are all recorded as 37 C.

As Table 1 shows, agar counts are comparable from only a little over half of the laboratories, and only when the results are obtained from the 37° plates. There is certainly no use in comparing figures from plates incubated 72 hours at 20° with those incubated 48 hours at 40°.

The composition of the agar appears to be fairly uniform, although with the several varieties of peptone now on the market there is some doubt as to this. Nineteen laboratories follow the procedures suggested in "Standard Method," one uses litmus-lactose agar and one adds 2% of peptone instead of the usual 1%.

The weight of opinion is quite strongly for agar counts after 24 hours at 37 C. Fourteen of the 24 laboratories use this method, while 9 of these use also the 48 hour count on gelatin at 20° and 2 use agar at 20°.

3. ORGANISMS OF THE TYPE B. COLI

Organisms of the type of B. coli are used generally as indicators of the sanitary quality of water supplies. A study of the procedures used in 23 laboratories for their detection discloses a great variety of practices. The different mediums used for the primary inoculation, together with the corresponding number of laboratories, is shown in Table 2.

TABLE 2

MEDIUMS FOR PRIMARY INOCULATIONS

Lactose broth	13
Lactose bile	8
Lactose and dextrose broths	1
Lactose agar and dextrose broth	1
Total	23

From Table 2 it is seen that 56.5% use lactose broth and 34.8% utilize a bile medium, although it has been shown by Jordan[1] and others that bile inhibits the growth of the colon bacillus and the medium has been dropped from "Standard Methods." In 2 of the 23 instances lactose-litmus-agar plates are used to check the broth results, and in one laboratory neutral red agar is used.

In 9 cases (about 40%) gas formation, in amounts of 10% or more, in the sugar medium after 24 or 48 hours is regarded as sufficient evidence of the presence of B. coli in the water, the actual numbers being figured from the number of tubes containing gas and the amount of water used. This is, of course, the so-called presumptive test. In 6 of the laboratories using this test lactose bile is used for the primary inoculation (2 using litmus-lactose-agar plates as a check), and the other 3 start with lactose broth (1 using neutral red agar plates as a check). In addition to the foregoing 9, in 3 laboratories the presumptive test is used on the raw water only but in conjunction with confirmatory tests on samples of treated water. Since it is well known that the presumptive test is likely to give high colon counts this procedure tends to give an exaggerated idea of the efficiency of the purification process. The argument used in favor of the presumptive test is "that previous experience has shown a large percentage of the tubes containing gas will give positive confirmatory tests."

The lactose broth used for the primary inoculation does not vary any more than results from the manufacture of the ingredients by different firms, but the lactose bile varies a great deal in respect to the source of the bile. In some laboratories fresh bile is used and in others dried preparations of various makes are utilized.

In making confirmatory tests for colon bacilli from the fermented broths 2 mediums are in use for the first isolation of organisms — Endo medium and lactose litmus agar.* Of the 14 laboratories in which confirmatory tests are used, 8 use Endo plates or streaks and 4 the lactose litmus agar. In one instance both mediums are used and in the other both are used but not on the same sample of water. The weight of opinion is decidedly in favor of Endo medium for this secondary inoculation.

Final diagnosis for colon bacilli by the appearance of colonies on Endo or lactose litmus agar is made in two instances, although in one

[1] Jour. Infect. Dis., 1913, 12, p. 326.

* In one case azolitmin is used. Since the reaction is the same as with litmus there is no reason to regard the medium as distinct for the purpose of isolating colon bacilli.

a doubtful result leads to transfers to test fermentation in dextrose and lactose broths.

In 3 cases the final test consists in the inoculation of lactose broth with organisms from characteristic colonies on Endo medium while in 4 more, colonies from Endo or lactose litmus agar are not only tested for lactose fermentation but are also examined for morphological characteristics after transferring to agar slants.[2]

This leaves 5 laboratories which favor further biochemical reactions for identification of colon bacilli. From one of these no statement was obtained concerning the exact procedure so that Table 3 shows the variety of tests employed in the other 4 cases and the number of times each test occurs. The only test on which there is entire agreement is that for gelatin liquefaction.

TABLE 3

FURTHER TESTS FOR B. COLI

Gelatin liquefaction	4
Indol production	3
Lactose fermentation	2
Nitrate reduction	2
Litmus milk reaction	1
Dextrose fermentation	1
Voges-Proskauer test	1
Methyl-red test	1

To summarize: only 4 of the 23 laboratories follow the so-called "completed test" as suggested in "Standard Methods" (characteristic colonies on Endo, formation of gas in lactose broth and demonstration of nonspore-forming organisms from an agar slant), 5 define colon bacilli in a more rigid manner than this, while the remaining 14 utilize a more liberal interpretation of the characteristics of that group. It is obvious that no proper comparisons of water supplies or of filter efficiencies should be made on such uncertain analytical data.

RELATION BETWEEN GELATIN AND AGAR COUNT

The relative value of agar and gelatin for water examinations has been a matter of some dispute. Those in favor of gelatin (20°) argue that the greater growth gives a more accurate estimate of filtration efficiencies while those preferring agar (37°) insist that it is more important to obtain an estimate of the number of organisms growing at body temperature and which are presumably of sewage origin.

[2] Am. Jour. Pub. Health, 1917, 7, p. 1050, gives a complete description of methods used in one laboratory.

There is an impression that the ratio of the gelatin to the agar count is about 10:1. In the replies to the questions on which this paper is based there is some evidence bearing directly on this point.* In Table 4 the ratio of gelatin counts (48 hours at 20°) to agar counts (24 hours at 37°) has been computed for raw and filtered waters from the data supplied by 5 filter plants. The average monthly counts for a year are combined to give an average yearly count and the ratio of gelatin to agar determined. In order to give an idea of the variations in this ratio and also of the actual numerical value of the counts, the highest and lowest found as monthly averages and the highest and lowest ratios based on these monthly average counts during the same years have been computed and are recorded in Table 5.

TABLE 4

RELATION OF GELATIN TO AGAR COUNTS. YEARLY DATA

	Yearly Average Count				Ratio Gelatin to Agar	
	Raw		Filtered			
	Gelatin	Agar	Gelatin	Agar	Raw	Filtered
1	1,770	580	36	9	3.3	4.0
2	58,300	5,200	149	10	11.1	14.9
3	5,880	388	10.7	4.7	15.1	2.3
4	4,872	1,236	46	56	3.9	0.8
5	36,000	650	13	3	55.5	4.3

TABLE 5

RELATION OF GELATIN TO AGAR COUNTS. MONTHLY DATA

	Monthly Average Count				Ratio Gelatin to Agar	
	Raw		Filtered			
	Gelatin	Agar	Gelatin	Agar	Raw	Filtered
1. High	4,100	1,546	97	15	10.8	12.1
Low	535	121	16	4	1.3	2.3
2. High	2,486,000	15,000	530	16	103.4	86.0
Low	11,200	6,000	14	5	1.2	1.1
3. High	12,700	1,110	44	8	53.0	11.0
Low	280	55	3	3	0.8	0.6
4. High	13,225	2,613	189	206	9.1	7.0
Low	511	362	6	2	0.9	0.2
5. High	72,000	4,400	35	7	338.0	19.0
Low	11,000	210	4	0	5.6	2.0*

* Omitting ratios involving 0 in the denominator.

Note: The ratios as given are those actually obtained for any one month and therefore have no relation directly to the counts given in the table.

An examination of Tables 4 and 5 shows no relation whatever to exist between the gelatin count at 20° and the agar count at 37°. There is, however, a tendency for the ratios to be lower in the filtered than

* See also Tanner, Univ. of Illinois Bull., Water Survey Series, 1916, 12, p. 242.

in the raw water, which indicates a larger relative removal of organisms growing at 20° compared to those growing at 37°, but further than this no definite statement can be made. There is no evidence in these figures that a 10:1 ratio is maintained.

SUMMARY

Statistics concerning the methods of water examination in connection with 24 purification plants in this country show that in no two instances are these methods exactly alike.

About two-thirds of the laboratories use gelatin as the medium for obtaining a total count at 20 C. The procedures with this medium are fairly consistent.

About seven-eighths of the laboratories use an agar medium for counts at some temperature, while 60% conform to a 24-hour count at 37 C. The composition of the medium is as consistent as possible with ingredients from varying sources.

Many differences are found in the methods for the detection of organisms of the type B. coli. About 60% of the laboratories use lactose broth and most of the remainder use lactose bile. In somewhat less than half of the instances the presumptive test only is made while the others use a great variety of confirmatory tests.

No definite relation is maintained between gelatin and agar counts.

In order to be comparable, data of water examinations must be collected under identical conditions. Since no uniformity in methods exists among the laboratories in this country, bacteriologic data of water supplies and water purification processes are not amenable to critical comparisons.